



Supplementary Materials for
**GPR15-mediated homing controls immune homeostasis in the
large intestine mucosa**

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Materials and Methods

Animals

Gpr15^{gfp/gfp} mice were generated by transfer of embryonic stem cells (E14 cells, 129SvEv) that were manipulated by homologous recombination. All the mice were kept in a specific pathogen free (SPF) facility. *Gpr15^{gfp/gfp}* mice used for the experiments were backcrossed to C57BL/6 at least 9 times unless specified. All comparisons were done between littermates or pups of littermates and mice were co-housed after weaning before the experiments. Mice used for *Citrobacter* infection were *SFB* (*Segmented Filamentous Bacteria*)-free. R.A. Flavell kindly provided us with *Foxp3^{ires-mrflp}* mice (19). Bone marrow of *Foxp3^{sf}*, *Ptprc^a* (*Scurfy*, *Ly5.1*) mice was kindly provided by A. Y. Rudensky (29). All animal studies were performed according to the protocol approved by the Institutional Animal Care and Usage Committee (IACUC) of New York University.

Cell preparation from intestines

We used a protocol modified from one described before (30). For the large intestine, cecum and colon were dissected from mice. We used Intramedic Polyethylene tubes (Becton Dickinson) to turn the tissues inside out exposing the epithelial layer during the process. Tissues were washed with ice-cold PBS once and treated sequentially with 1mM DTT/PBS for 10 min and with 30mM EDTA/PBS for 10 min twice while shaking. After these treatments, samples were washed once with PBS and the polyethylene tube was removed. Cecal or colonic patches were cut out and the rest of the tissues were digested with collagenase 8 (Sigma), Dispase (Worthington), and DNase I in RPMI-10 (RPMI media supplemented with 10% Fetal Bovine Serum (FBS), Penicillin/Streptomycin, 2mM L-glutamine, 1mM Sodium Pyruvate, 10mM Hepes, 100μM non-essential amino acids, and 55μM β-mercaptaethanol) for 1.5-2h at 37°C until digestion was complete. Digested tissues were filtered through a 100μm filter and cells were recovered at the interface between 40% and 80% percoll (GE healthcare) after spinning for 20 min at 850g. Later, samples were filtered twice with 40μm Nylon mesh (Fisher Scientific) and either stained for cellular markers or stimulated before intracellular staining. Cell numbers were counted by Vi-Cell XR (Beckman Coulter) or by AccuCount Fluorescent particles (Spherotech, Inc) during flow cytometry analysis.

For the small intestine preparation, the whole small intestine was taken from mice and Peyer's patches were removed. The rest of the tissues were cut longitudinally and eventually cut into 8 pieces. Subsequently, samples were washed with ice-cold PBS once and treated with 5mM EDTA/PBS for 10 min while shaking. Then, they were washed in PBS twice. Tissues were digested in Collagenase D (Roche), Dispase (Worthington), and DNase I in RPMI-10 for 1.5-2h at 37°C. Digested tissues were processed as described above.

Gene Chip Analysis

RNA was prepared from sorted GFP⁺CD4⁺ T cells and GFP⁻CD4⁺ T cells in LILP of *Gpr15^{gfp/+}* mice. For microarray analysis, GeneChip Mouse Genome 430 2.0 arrays

(Affymetrix) were used according to the manufacturer's protocols by staff at the Genome Technology Center (GTC) in the NYU Medical Center. Data were analyzed with GeneSpring GX11.5 software. Genes with p-values no more than 0.05 and with more than 1.6-fold change were selected. Fold change shown in the table was based on expression in GFP⁺CD4⁺T cells compared to that in GFP⁻CD4⁺T cells.

Intracellular cytokine staining and FOXP3 staining of lymphocytes from the gut

For intracellular cytokine staining, single cell suspensions prepared from the small or large intestines were stimulated in the presence of Monensin (BD GolgiStop) with PMA (50ng/ml, Sigma) and Ionomycin (500ng/ml, Sigma) in RPMI-10 for 4h. Subsequently, cells were stained for cellular markers and additionally stained by fixable dye for dead cells (Invitrogen). Cells were fixed and permeabilized with BD Cytofix/Cytoperm plus (BD Biosciences) according to the manufacturer's protocol. For FOXP3 and HELIOS staining, single cell suspensions prepared from intestines were stained for cellular markers and later fixed and permeabilized using the FOXP3/Transcription factor buffer set (eBiosciences) according to the manufacturer's protocol.

Short-term competitive homing assay

The coding region of mouse GPR15 was cloned into the MSCV-IRES-Thy1.1 retroviral construct (31). Retroviruses were generated by transient transfection of Phoenix cells. CD4⁺CD62L^{high}CD44^{low}CD25⁻ T_{naïve} cells were sorted by FACS Aria IIu (BD Biosciences) and stimulated with 0.25µg/ml of αCD3ε (145-2c11, ATCC) and 1µg/ml of αCD28 (37.51, eBiosciences) antibodies cross-linked by plate-bound α-hamster IgG (MP Biochemical) in RPMI-10. At day 1 and day 2 after stimulation, cells were transduced with retrovirus (spinning at 850g for 2h with 4µg/ml of polybrene). CD4⁺ T_{naïve} cells from B6.SJL-*Ptprca^aPep3^b*/BoyJ (The Jackson Laboratory) were used for transduction of *Gpr15* and CD4⁺ T_{naïve} cells from C57BL/6 were used for transduction of empty vector. At day 3, cells were washed and plated again only with 100 U/ml of IL-2. At day 5, live cells were harvested by lymphocyte-M (Cedarlane). The congenic cells were mixed at a 1:1 ratio and 20-30 million mixed cells in total were transferred intravenously into the recipient mice (C57BL/6). Migration of donor cells into each organ was determined at 10h after transfer (unless specified) by flow cytometry analysis. All donor cells expressed THY1.1. GPR15-expressing cells and control cells expressed CD45.1 and CD45.2, respectively.

For blocking integrin function, 100µg of Rat IgG2a,k isotype control antibody (Biolegend), anti-integrin β7 antibody (Biolegend, FIB504), anti-integrin α4 antibody (Millipore, PS/2), or 100µg each of FIB504 and PS/2 were injected into recipient mice 12h before transfer.

For examining the activity of GPR15 ligand, competitive homing assays were performed with Germ-free mice or antibiotics-treated (with mixture of ampicillin, vancomycin, metronidazole, and neomycin for 2 weeks) mice as recipients.

For Het and KO competitive homing assay, CD4⁺ T_{naïve} cells (CD62L^{hi}CD44^{lo}CD25⁻mRFP⁻ GFP⁻) were sorted from *Gpr15^{gfp/+} Foxp3^{ires-mrfp}* or *Gpr15^{gfp/gfp} Foxp3^{ires-mrfp}* mice which have different congenic markers. Sorted cells were stimulated with 0.25µg/ml αCD3ε (145-2c11, ATCC) and 1µg/ml of αCD28 antibodies (37.51, eBiosciences) cross-linked by plate-bound α-hamster IgG in the presence of human IL-2 (100U/ml), IL-21 (25ng/ml, R&D systems), human TGF-β1 (5ng/ml, Peprotech), and retinoic acid (100pM) for 3 days. At day 3, cells were washed and plated again only with human IL-2 (100U/ml), IL-21 (25ng/ml), human TGF-β1 (5ng/ml), and retinoic acid (100pM). At day 5, the congenic cells were mixed at a 1:1 ratio and ~80 million mixed cells in total were transferred intravenously to the congenic recipient mice.

Antibiotics treatment

For antibiotics treatment, a mixture of ampicillin (1g/L), vancomycin (0.5g/L), metronidazole (1g/L), and neomycin (1g/L) was provided in the drinking water to breeding pairs, and newborn pups were continuously supplied with this mixture after weaning until the analysis.

OVA feeding

1.5% of chicken ovalbumin (Sigma) was added to drinking water for OT-II transgenic, *Rag2^{-/-}* mice with *Gpr15^{gfp/+}* or *Gpr15^{gfp/gfp}* genotypes. OVA-containing drinking water was replaced every other day. 10 days later, cells from different organs were prepared and examined for GFP expression and cell numbers.

In vitro stimulation of T cells to examine GFP induction

All T cell cultures were done in RPMI-10. CD4⁺ T_{naïve} (CD62L^{hi}CD44^{lo}CD25⁻mRFP⁻ GFP⁻), Tregs with naïve phenotype (CD62L^{hi}CD44^{lo}CD25⁺mRFP⁺ GFP⁻), T_{memory} (CD62L^{lo}CD44^{hi}CD25⁻mRFP⁻ GFP⁻), and Tregs with memory phenotype (CD62L^{lo}CD44^{hi}CD25⁺mRFP⁺ GFP⁻) were sorted from *Gpr15^{gfp/+} Foxp3^{ires-mrfp}* mice. Sorted cells were stimulated with 0.25µg/ml αCD3ε (145-2c11, ATCC) and 1µg/ml of αCD28 antibodies (37.51, eBiosciences) cross-linked by plate-bound α-hamster IgG in the presence of human IL-2 (100U/ml) alone, or additionally with IL-21 (10ng/ml, R&D systems) or IL-6 (20ng/ml, Peprotech), in the presence of varying concentrations of human TGF-β1 (αTGFβ blocking antibody [R&D systems], or 0, 0.5ng/ml, or 5ng/ml TGF-β1 [Peprotech]) for 3 days. The same conditions were used for retinoic acid treatment with IL-2 only.

In vitro Treg-mediated suppression assay

We used a slightly modified condition from that described previously (32). Tregs (CD4⁺CD25⁺mRFP⁺) were sorted from *Gpr15^{+/+} Foxp3^{ires-mrfp}* or *Gpr15^{gfp/gfp} Foxp3^{ires-mrfp}* mice. CD4⁺ T_{naïve} cells (CD4⁺CD62L^{hi}CD44^{lo}CD25⁻) were sorted from B6.SJL-*Ptprc^aPep3^b*/BoyJ mice (The Jackson Laboratory) and labeled with 1.25µM of CFSE (Invitrogen) for 15min at 37°C, by making 2.5µM of CFSE in RPMI/1% FBS and adding the same volume of cells in RPMI/1% FBS during vortexing. Labeling was stopped by adding 5 volumes of ice-cold FBS. In addition, splenocytes obtained from

C57BL/6 mice were treated with 50µg/ml of Mitomycin C (Sigma) for 40 min at 37°C. 8×10^4 splenocytes (Mitomycin C-treated) were mixed with 3×10^4 $T_{naïve}$, varying numbers of Tregs, and 1µg/ml of αCD3ε (145-2c11) in RPMI-10 in each well of 96 well round bottom plates. At day 3, proliferation of CD45.1⁺ $T_{naïve}$ was examined by flow cytometry analysis.

Mixed bone-marrow chimera generation

Male *Gpr15^{gfp/gfp}* mice were irradiated twice with 600 rad and i.v. transferred with a 1:1 mixture of Thy1-depleted bone marrow cells from *Gpr15^{+/+}* and *Foxp3^{sf}* mice or from *Gpr15^{gfp/gfp}* and *Foxp3^{sf}* mice (2×10^6 cells total). Chimeric mice were infected with *Citrobacter rodentium* 7 weeks later.

Citrobacter-induced colitis

Citrobacter rodentium strain DBS100 (ATCC 51459; American Type Culture Collection) was recovered by inoculation in 5ml LB broth and shaking at 37°C overnight. The next day, the bacteria was inoculated in fresh medium (500ml) and let grow until the culture reached the exponential phase ($OD_{600}=0.4-0.6$). Mice were infected by oral gavage with 8×10^9 of *C. rodentium* in a total volume of 400µl per mouse ($O.D_{600} = 2.5 \times 10^8$ bacteria). The concentration of bacteria was confirmed by plating serially diluted cultures on MacConkey agar plates. Colonic tissues were analyzed at day 9 or day 10 after the infection by H&E staining, RT-PCR, and colony forming assay. Paraffin embedding and H&E staining were performed by staff of the Histopathology core at the NYU Medical Center.

αCD40 antibody-induced inflammation/colitis and rescue by Treg transfer

Injection of agonist αCD40 (FGK 45) antibody induces acute systemic and local inflammatory diseases, including wasting, splenomegaly, hepatopathology, and colitis in *Rag1* or *2* deficient mice which do not have adaptive immunity (23). To evaluate the inflammatory reactions upon αCD40 antibody injection in mice with adaptive immunity, *Gpr15^{gfp/+}* and *Gpr15^{gfp/gfp}* mice were administered 200-300µg of αCD40 antibody intra-peritoneally and inflammatory cytokine expression was examined by RT-PCR in different tissues at day 3. For induction of acute colitis, *Rag2^{-/-}* mice were injected with αCD40 antibody (200µg i.p.). 7 days later when the pathology reached its peak, colonic tissues were examined. It has been shown that transfer of Tregs can rescue colitis induced in *Rag2^{-/-}* mice (18, 24, 25). For the rescue, *Rag2^{-/-}* mice were transferred intravenously with 5×10^5 CD4⁺CD25⁺mRFP⁺ Tregs either from *Gpr15^{+/+}Foxp3^{ires-mrfp}* mice or *Gpr15^{gfp/gfp}Foxp3^{ires-mrfp}* mice. 3 weeks later, mice were injected with αCD40 antibody (200µg i.p.) and colonic tissues were examined at day 7 after antibody injection. Tissues were processed for H&E staining as described above.

Colitis induction by $T_{naïve}$ transfer

James G. Fox kindly provided us with *Helicobacter hepaticus* (33). *Rag2^{-/-}* mice were infected by *Helicobacter hepaticus* as described before (24) and transferred intravenously with 3×10^5 CD4⁺ $T_{naïve}$ cells either from *Gpr15^{+/+}Foxp3^{ires-mrfp}* mice or

Gpr15^{gfp/gfp}Foxp3^{ires-mrfp} mice. Colonic tissues were examined at six weeks after the transfer. Tissues were processed for H&E staining as described above.

Histology scoring of H&E stained slides

The H&E slides from each sample were examined double-blindly by a team of a gastrointestinal pathologist and an investigator. The histology scoring is based upon evaluation of 8 different criteria. For each case, 3 foci of the most severely affected microscopic fields were chosen for analysis and the score for each criterion was an average from three foci. The final score is a combination of those from each criterion. Scoring was performed under high power view (40X) as follows: Neutrophil infiltration (0: None, 1: 1-50 cells, 2: 50-100 cells, 3: 100+ cells); Edema (0: None, 1: Mild, 2: Moderate, 3: Severe); Goblet cell depletion (0: 50+/HPF, 1: 25-50/HPF, 2: 10-25/HPF, 3: <10/HPF); Crypt damage (0: Intact, 1: Basal 1/3, 2: Basal 2/3, 3: Entire loss); Atrophy & Crypt loss (0: Normal crypt, 1: Mild, 2: Moderate, 3: Severe); Epithelial regeneration (0: Complete, 1: Slight injury, 2: Surface not intact, 3: No tissue repair); Epithelial hyperplasia (0: None, 1: 1-50%, 2: 51-100%, 3: >100%); Erosion & Ulceration (0: None, 1: Focal, lamina propria, 2: Muscularis propria, 3: Full thickness). Acute inflammation index was a combined score of neutrophil infiltration and Edema.

Reverse transcription and real-time PCR

Cells were resuspended in Trizol (Invitrogen) and processed according to the manufacturer's protocol to prepare RNA. Subsequently, RNA samples were treated with RNase-free DNase (Roche) and reverse-transcribed by Superscript III (Invitrogen) or by First-Strand cDNA Synthesis Kit (Affymetrix) with poly-T primer. For detection of mRNA, we used Taqman Gene Expression Assays (Applied Biosystems), platinum Taq polymerase (Invitrogen), and LightCycler 480II (Roche). Relative expression level was normalized to *Beta-actin*. Assay IDs for Taqman probe/primer set are as follows. *Gpr15*: Mm01346276_g1; *Beta-actin*: Mm02619580_g1; *Tnfalpha*: Mm00443258_m1; *Il-1beta*: Mm01336189_m1; *Cxcl2*: Mm00436450_m1; *BETA-ACTIN*: Hs99999903_m1; *GPR15*: Hs00922903_s1.

Human samples

Blood lymphocytes were prepared from IRB-approved buffy coats from the New York Blood Center. Buffy coat was first spun with Ficoll for 30 min at 850g. Subsequently, CD4⁺ cells were enriched with AutoMACS purification (Miltenyi Biotec) and later sorted. Anonymized, grossly normal samples of colon or ileum were obtained from colon cancer resection specimens. Samples of normal duodenum were obtained following the Whipple procedure for pancreatic cancer resection. All human gut tissues were acquired by the NYULMC Biorepository Core. Muscularis and fat layer were removed first and washed with ice-cold PBS once. Later, samples were treated sequentially with 1mM DTT/PBS for 10 min and with 30mM EDTA/PBS for 10 min twice. After these treatments, samples were washed once with PBS and extra fat tissue was removed again. The lamina propria layer was digested with collagenase 8 (Sigma) and Dnase I in RPMI-10 for 1.5-2hrs at 37°C until digestion was complete. Digested tissues were filtered through a 100µm filter

and cells were recovered at the interface between 40% and 80% percoll (GE healthcare) after spinning for 20 min at 850g. CD127^{lo}CD25^{hi}CD4⁺CD3⁺ cells and CD127^{hi}CD25^{lo}CD4⁺CD3⁺ cells were sorted for enrichment of Tregs and non-Tregs, respectively, and their enrichment was confirmed by RT-PCR (*FOXP3*: Hs03987537_m1, Applied Biosystems).

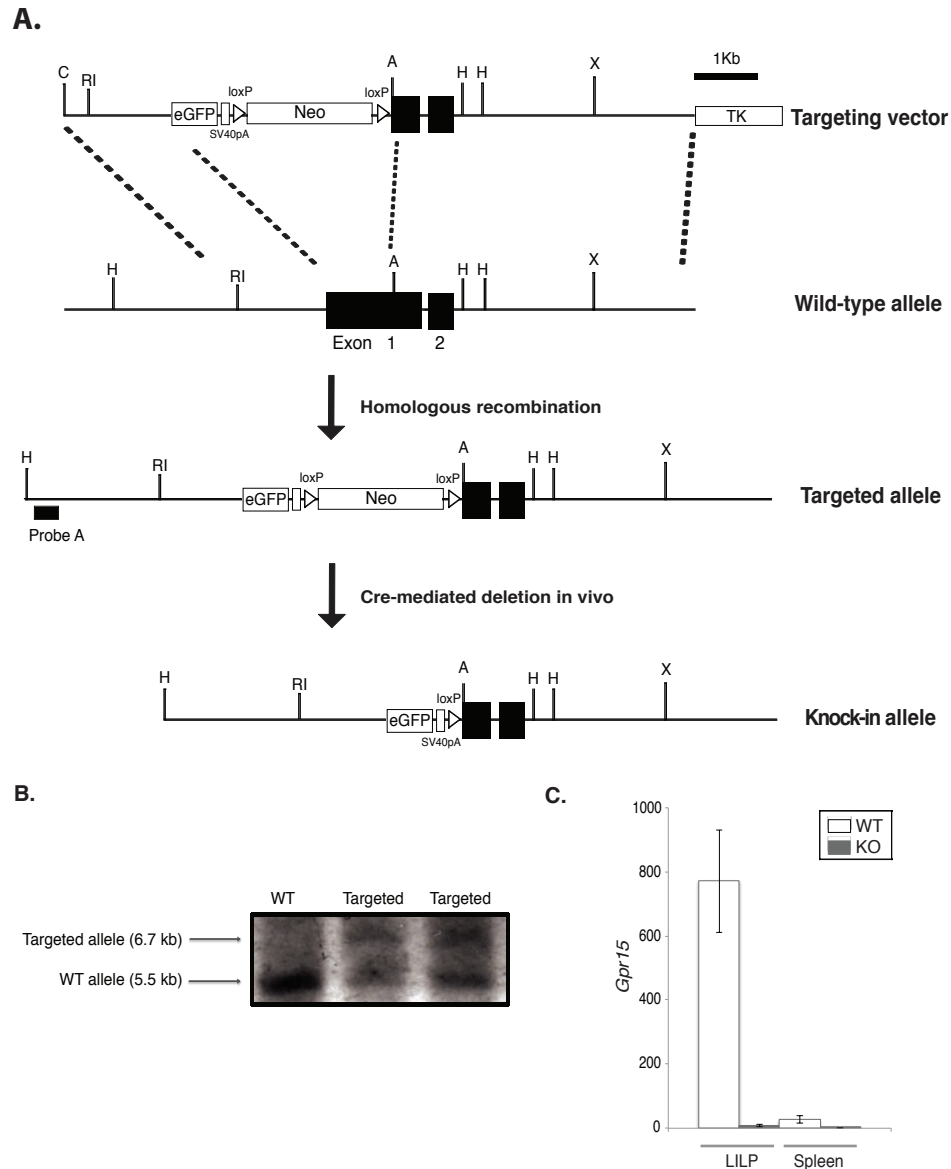


Fig. S1. Generation of *Gpr15* GFP knock-in mice. **(A)** Homologous recombination strategy for generating the mutant allele. Deletion of the Neo gene was achieved by Cre-mediated germline deletion in vivo (A: ApaI; C: ClaI; RI: EcoRI; H: HindIII; X: XbaI). **(B)** Southern blot of wild-type and targeted allele to confirm homologous recombination (Probe A with HindIII digestion) **(C)** Taqman RT-PCR of *Gpr15* mRNA in TCR β^+ cells, normalized to *Beta-actin*.

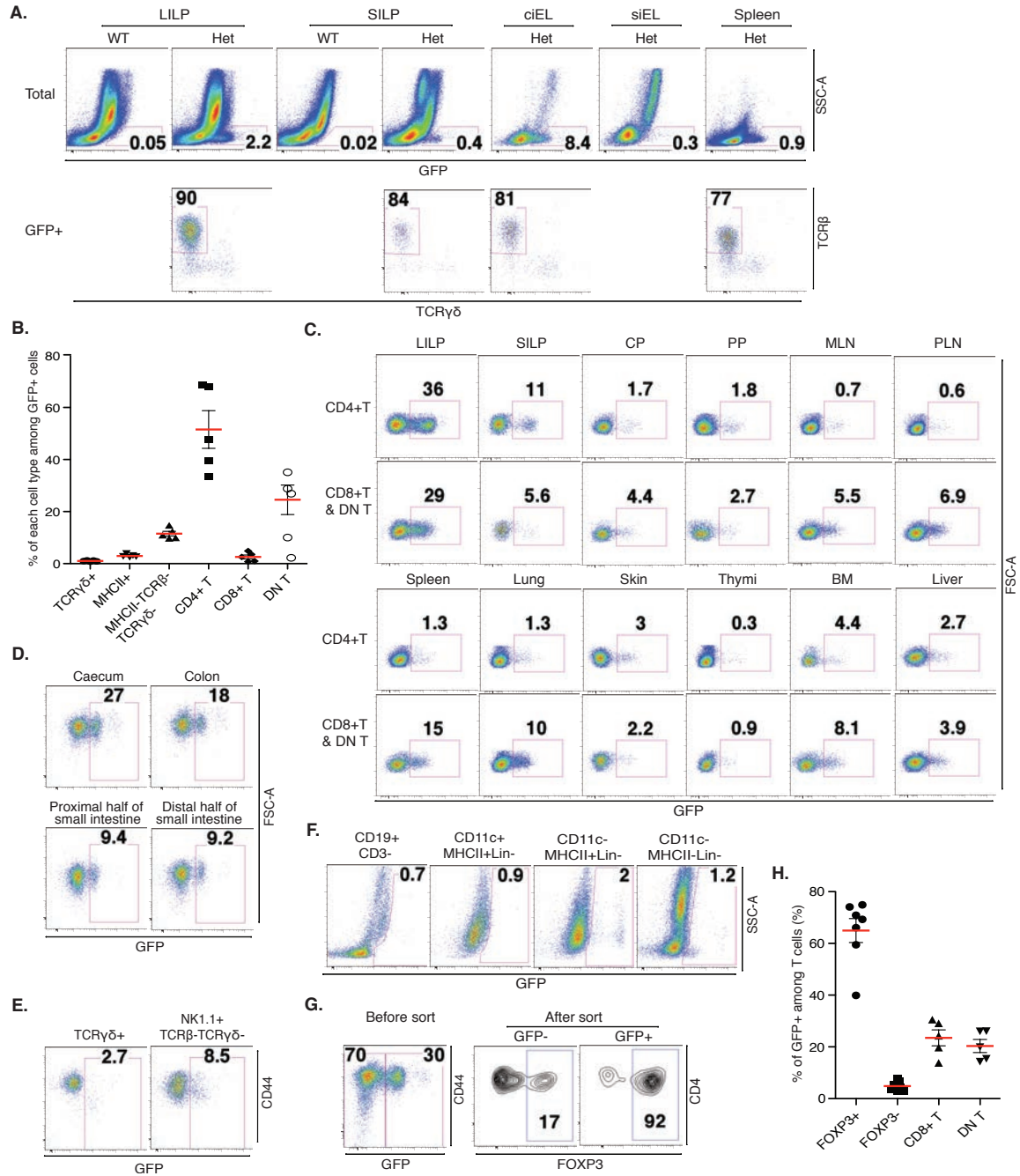


Fig. S2. GPR15 is preferentially expressed in LILP Tregs. **(A)** GFP expression in different tissues of *Gpr15^{gfp/+}* mice. GFP⁺ populations were additionally analyzed for T cell receptor expression. Most GFP⁺ cells were TCRβ⁺. **(B)** Cellular identity of GFP⁺ population was determined by surface staining (DN T: CD4⁺CD8β⁻T cells). CD4⁺ T cells made up the most significant population of GFP⁺ cells in the LILP (*B6/Balb/c* mixed background). **(C)** GFP expression was determined among CD4⁺ T cells, CD8⁺ T, and DN T cells from various tissues of *Gpr15^{gfp/+}* mice. T cells from the LILP have the most significant expression of GFP. **(D)** Different parts of the large intestine and the small intestine were examined for GFP⁺ cells among CD4⁺ T cells. **(E-F)** GFP expression in

various immune cell subsets from the LILP: TCR $\gamma\delta^+$ (TCR $\gamma\delta^+$ T cells); NK1.1 $^+$ TCR β^- , TCR $\gamma\delta^-$ (NK cells); CD19 $^+$ CD3 $^-$ CD45 $^+$ (B cells); CD11c $^+$ MHCII $^+$ Lin $^-$ CD45 $^+$ (Dendritic cells); CD11c $^-$ MHCII $^+$ Lin $^-$ CD45 $^+$ (Monocytes); CD11c $^-$ MHCII $^-$ Lin $^-$ CD45 $^+$ (Granulocytes and others). **(G-H)** GFP $^+$ and GFP $^-$ cells among CD4 $^+$ T cells in the LILP of *Gpr15^{gfp/+}* mice (*B6/Balb/c* mixed background) were sorted and stained for FOXP3. Percentage of GFP $^+$ cells in each T cell subset from the LILP is shown **(H)**. GFP $^+$ cells are most abundant among FOXP3 $^+$ regulatory T cells (n=5-7).

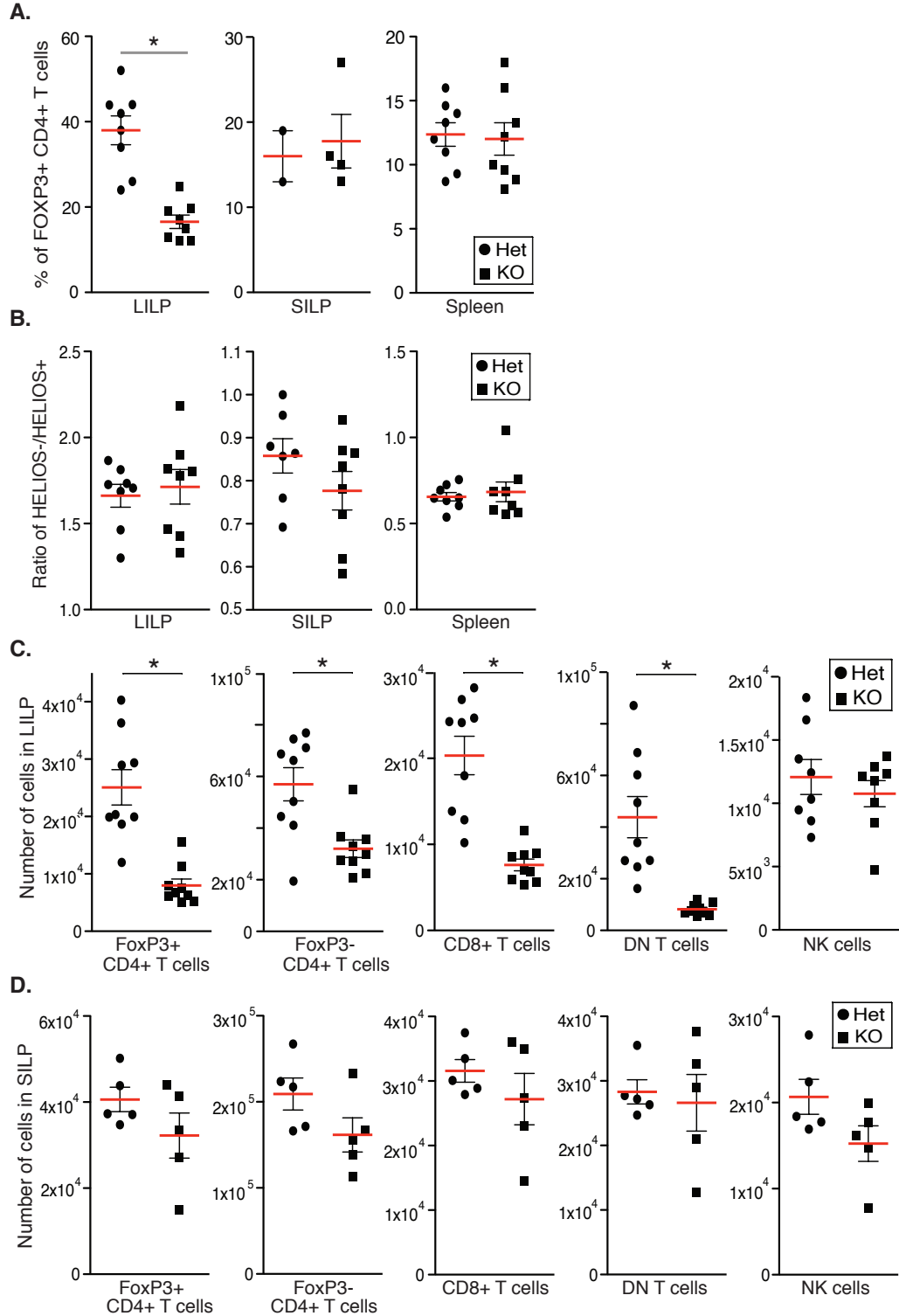


Fig. S3. Effect of GPR15 deficiency on intestinal lymphocyte subsets. **(A)** Percentage of FOXP3+ Tregs among CD4 T cells in different tissues of *Gpr15^{gfp/+}* mice (Het) and *Gpr15^{gfp/gfp}* (KO) mice (129/B6 mixed background: n=2-8, combined from at least two independent experiments). **(B)** Ratio of HELIOS⁻/HELIOS⁺ cells among Foxp3⁺ T cells in the LILP, SILP, and spleen of Het and KO mice. HELIOS was used as a marker for

thymus-derived Tregs. **(C-D)** Cell numbers for each immune cell subset (FOXP3⁺CD4⁺ Tregs, FOXP3⁺CD4⁺ T cells, CD8⁺ T cells, DN T (CD4⁺CD8⁺ T cells), and NK cells) in the LILP **(C)** and SILP **(D)** of Het and KO mice (n=9 for LILP, n=5 for SILP). Representative of at least three independent experiments. *p<0.05 (t-test).

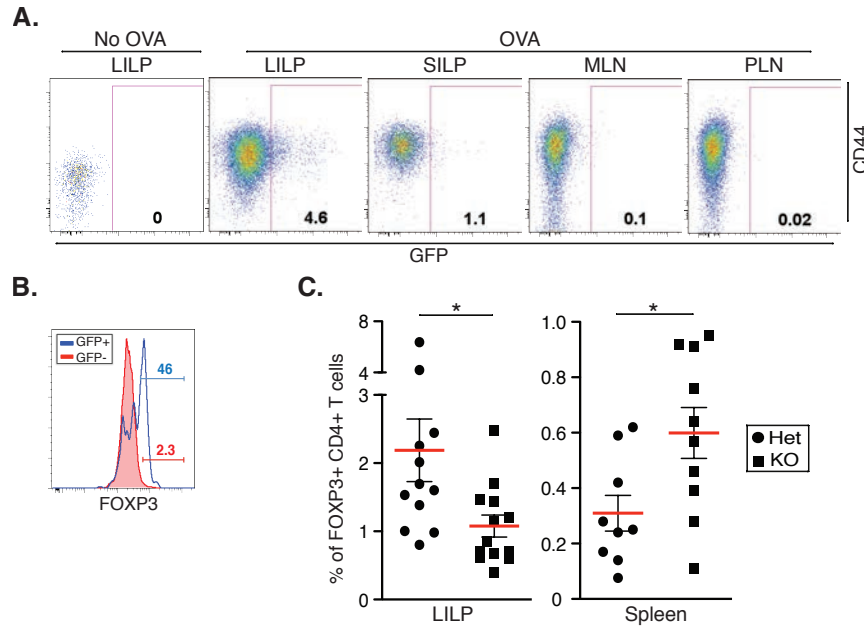


Fig. S4. Treg frequency in the LILP decreases in the absence of GPR15 in an antigen-specific immune response. **(A-B)** 1.5% Ovalbumin (OVA) in drinking water was provided to OT-II *Rag2*^{-/-} *Gpr15*^{gfp/+} mice. **(A)** GFP expression in T cells from various tissues (Average percentage of GFP⁺: 4.5(±0.2)% for LILP; 0.06 (±0.02)% for MLN, 0.02(±0.01)% for PLN (n=3), Representative of three independent experiments). **(B)** GFP⁺ and GFP⁻ cells in the LILP were sorted and stained for FOXP3. **(C)** Percentages of FOXP3⁺ cells among CD4⁺ T cells in LILP (left panel) and spleen (right panel) of OT-II *Rag2*^{-/-} *Gpr15*^{gfp/+} (Het) and OT-II *Rag2*^{-/-} *Gpr15*^{gfp/gfp} (KO) mice (n=9-13, combined result of three independent experiments). * p<0.05 (t-test).

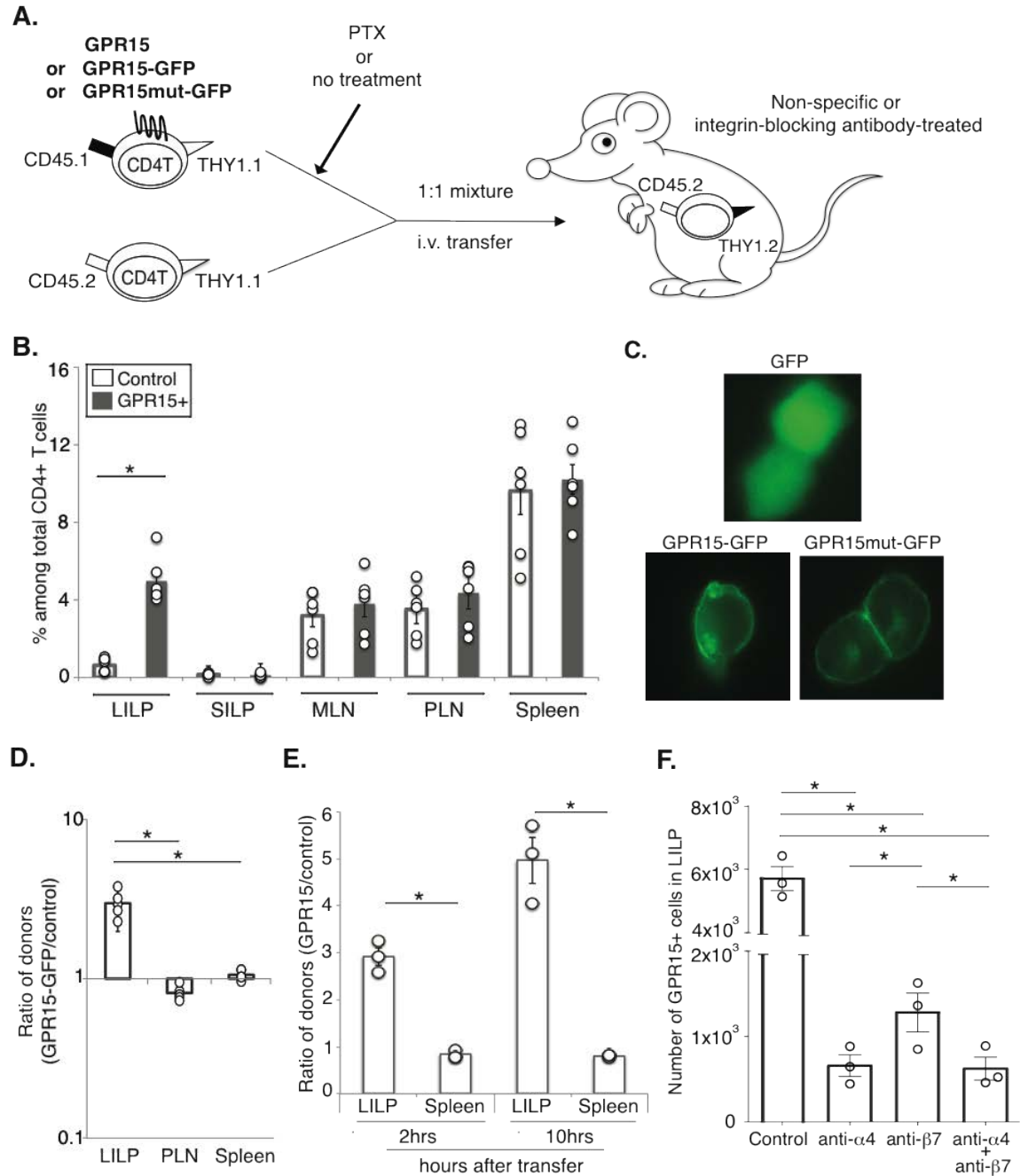


Fig. S5. GPR15-mediated signaling provides a selective advantage for homing to the large intestine in cooperation with integrin α 4 and β 7. **(A)** A diagram of short-term competitive homing assay with CD4⁺ T cells transduced with various forms of GPR15 performed in Fig. 2A-C, and Fig. S5B, S5D-F, S9B-C. *Gpr15*-transduced and control-transduced cells express a common marker, THY1.1, and different congenic markers, CD45.1 and CD45.2, respectively **(B)** Percentage of *Gpr15*-transduced and control-transduced donor cells among total CD4⁺ T cells (cells in the recipients included) in

different tissues (SILP: small intestine lamina propria; MLN: Mesenteric lymph nodes; PLN: Inguinal, Brachial, and Axillary lymph nodes) (n=6, combined result of three independent experiments). (C) Cell surface expression of GPR15-GFP and GPR15mut-GFP was confirmed in 293T cells. (D) Homing of CD4⁺ T cells transduced with *Gpr15* fused with *gfp* (GPR15-GFP) compared with those transduced with control vector (n=6, combined result of four independent experiments). (E) Time course experiment after transfer of *Gpr15*-transduced and control-transduced donor CD4⁺ T cells. GPR15⁺ cells display preferential migration to the LILP at 2h after transfer (n=3). (F) Recipient mice were injected i.p. with 100μg of non-specific or integrin-blocking antibodies 12h before transfer. *Gpr15*-transduced and control-transduced donor CD4⁺ T cells were also treated with corresponding antibodies before transfer. Numbers of GPR15⁺ cells in the LILP are shown (n=3, a representative of two-independent experiments). *p<0.05 (t-test).

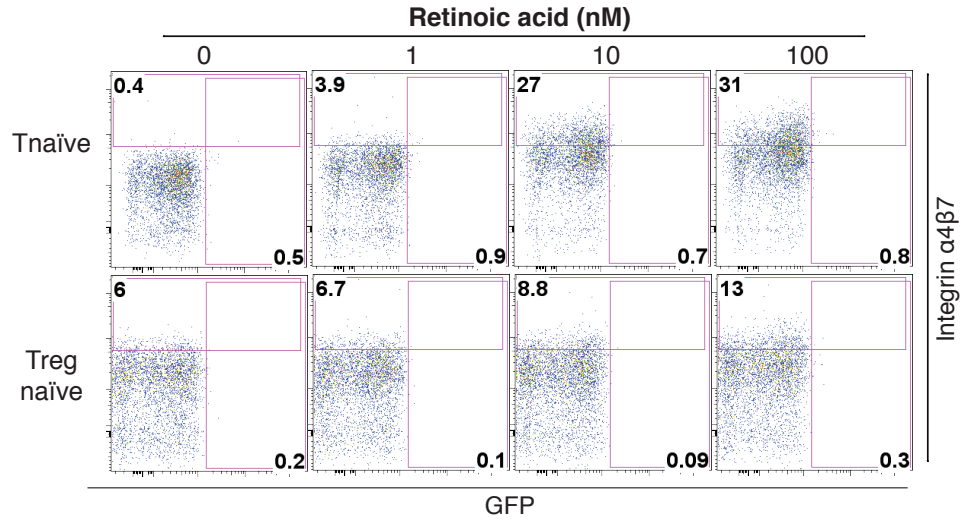


Fig. S6. Retinoic acid does not induce GPR15 expression in vitro. $CD4^+ GFP^- T_{naïve}$ and Tregs with naïve phenotype ($CD62L^{hi}$, $CD44^{lo}$) were sorted from $Gpr15^{gfp/+} Foxp3^{ires-mrfp}$ mice and stimulated in the presence of various concentration of retinoic acid for 3 days.

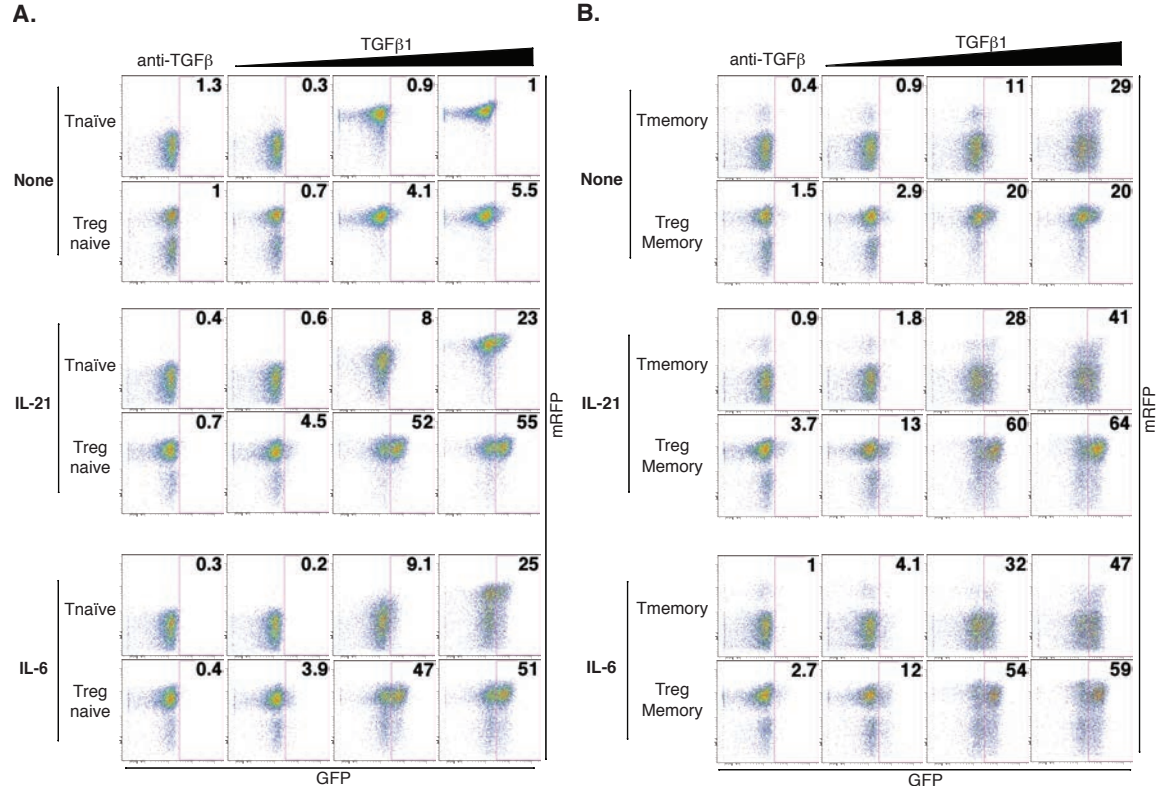


Fig. S7. GPR15 can be effectively induced in Treg cells and memory T cells in vitro. $CD4^+ GFP^- T_{naive}$ and Tregs with naive phenotype ($CD62L^{hi}$, $CD44^{lo}$) or with memory phenotype ($CD62L^{lo}$, $CD44^{hi}$) were sorted from $Gpr15^{gfp/+} Foxp3^{ires-mrpf}$ mice and stimulated in the presence of IL-2 (100U/ml) alone, or additionally with IL-21 (10ng/ml) or IL-6 (20ng/ml) and varying concentration of TGF-β1 (α TGF-β blocking antibody, or 0, 0.5ng/ml, 5ng/ml of additional TGF-β1). Expression of the GPR15 and Foxp3 reporters was examined at day 3 (Representative of four independent experiments).

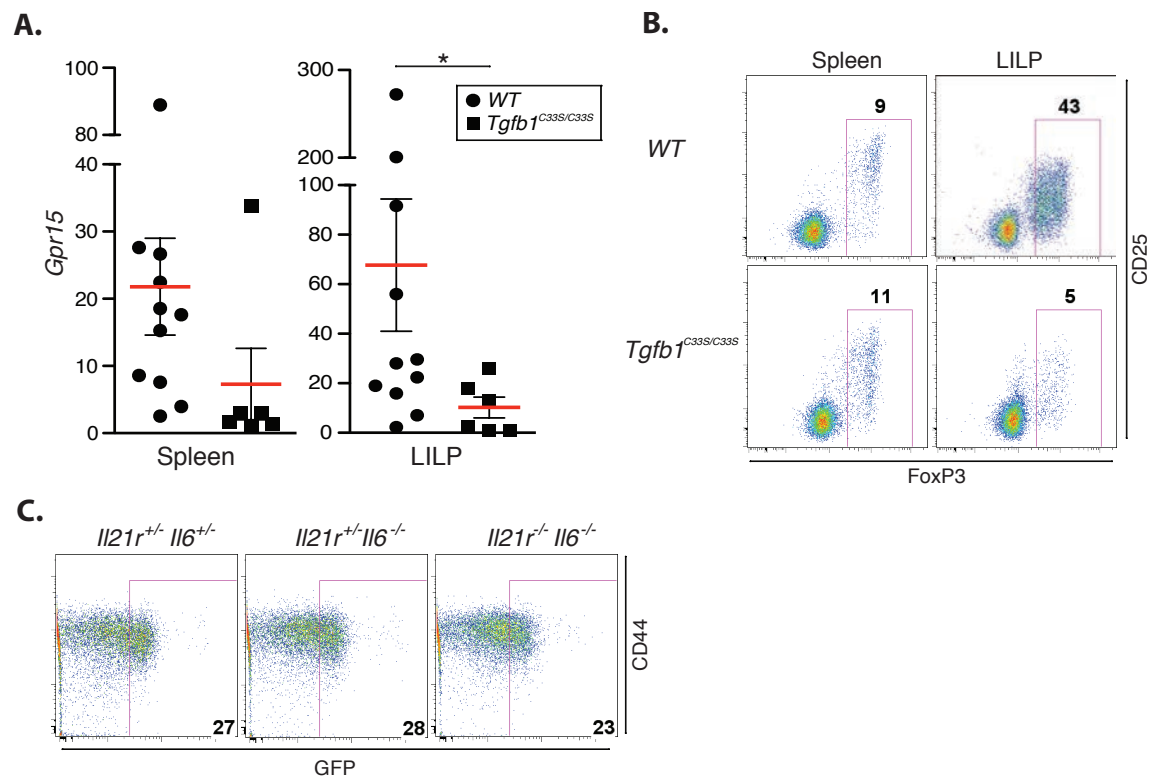


Fig. S8. GPR15 expression is dependent on TGF- β 1 but not on IL-21 or IL-6 in vivo. **(A)** Taqman RT-PCR for *Gpr15* mRNA in T cells from spleen and LILP of *Tgfb1*^{C33S/C33S} mutant (KI) and wild-type littermate mice (WT), normalized to *Beta-actin* (n=11(WT), 6(KI), combined results of three independent experiments). **(B)** FOXP3⁺ Treg frequency was examined in spleen and LILP of *Tgfb1*^{C33S/C33S} mutant and wild-type littermate mice (WT). GPR15 expression was reduced in spleen even with a similar frequency of Tregs in KI compared to WT mice. **(C)** *Gpr15*^{gfp/+} mice were crossed to *Il6*^{-/-} *Il21r*^{-/-} mice to determine the in vivo role of IL-21 and IL-6 on *Gpr15* expression. *Il6*^{-/-} *Il21r*^{-/-} mice had a similar amount of GFP expression as control mice. *p<0.05 (t-test).

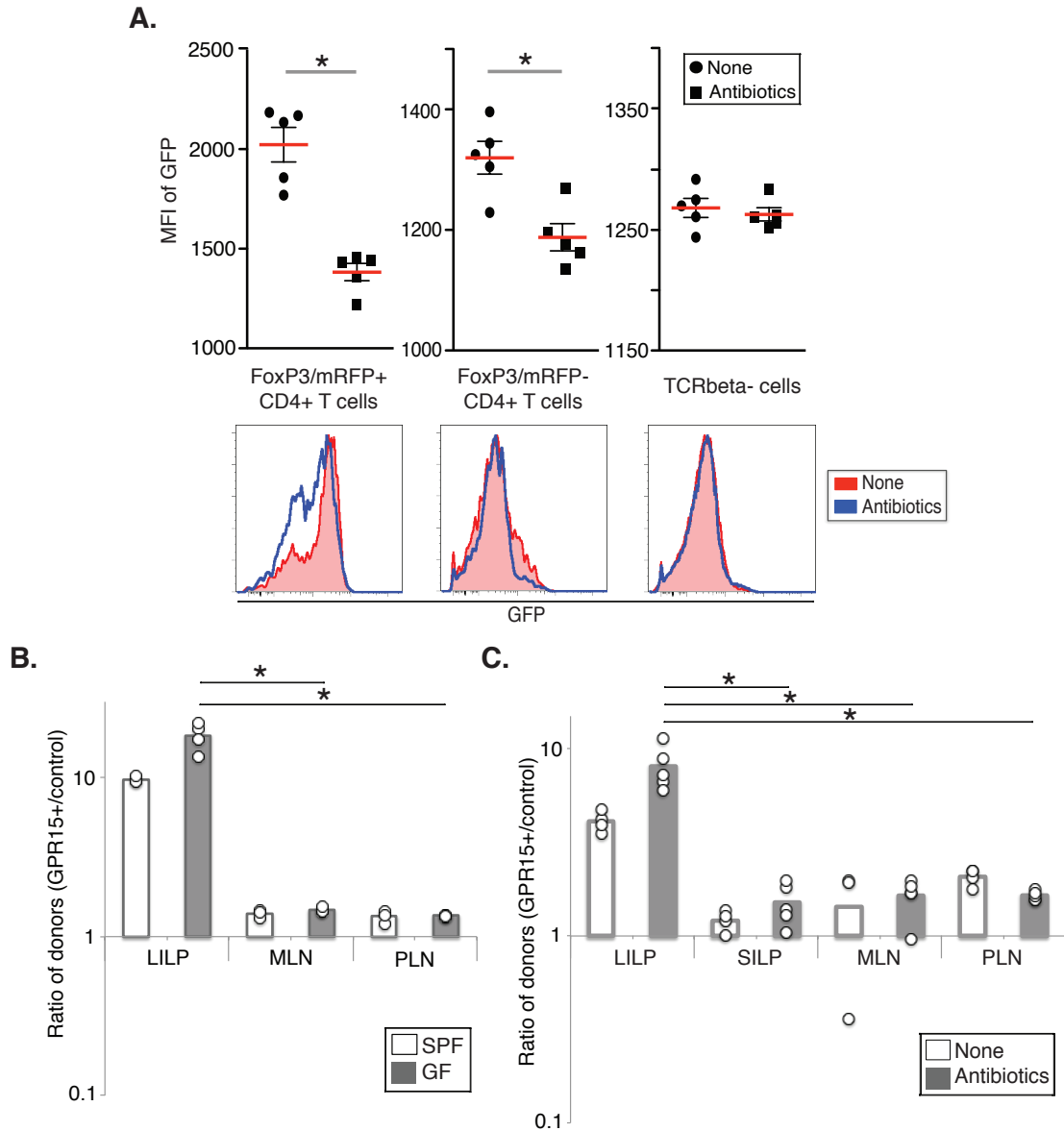


Fig. S9. Gut microbiota promotes GPR15 expression, but is not required for production of chemoattractant. **(A)** *Gpr15^{gfp/+}*, *Foxp3^{ires-mrfp}* mice were treated with a combination of antibiotics in the drinking water from birth. Mean fluorescence intensity (MFI) of GFP was examined in each cell type from the LILP (n=5, representative of three independent experiments). Representative histograms for each cell type are shown below. **(B-C)** Competitive homing assays were performed as described in fig. S5A. **(B)** Specific pathogen-free (SPF) or germ-free (GF) mice were used as recipients. **(C)** Specific pathogen-free mice (None) or mice treated with a combination of antibiotics were used as recipients. GPR15⁺ cells preferentially homed to the LILP in GF and antibiotics-treated mice, indicating that GPR15 ligand production is not dependent on gut microbiota.

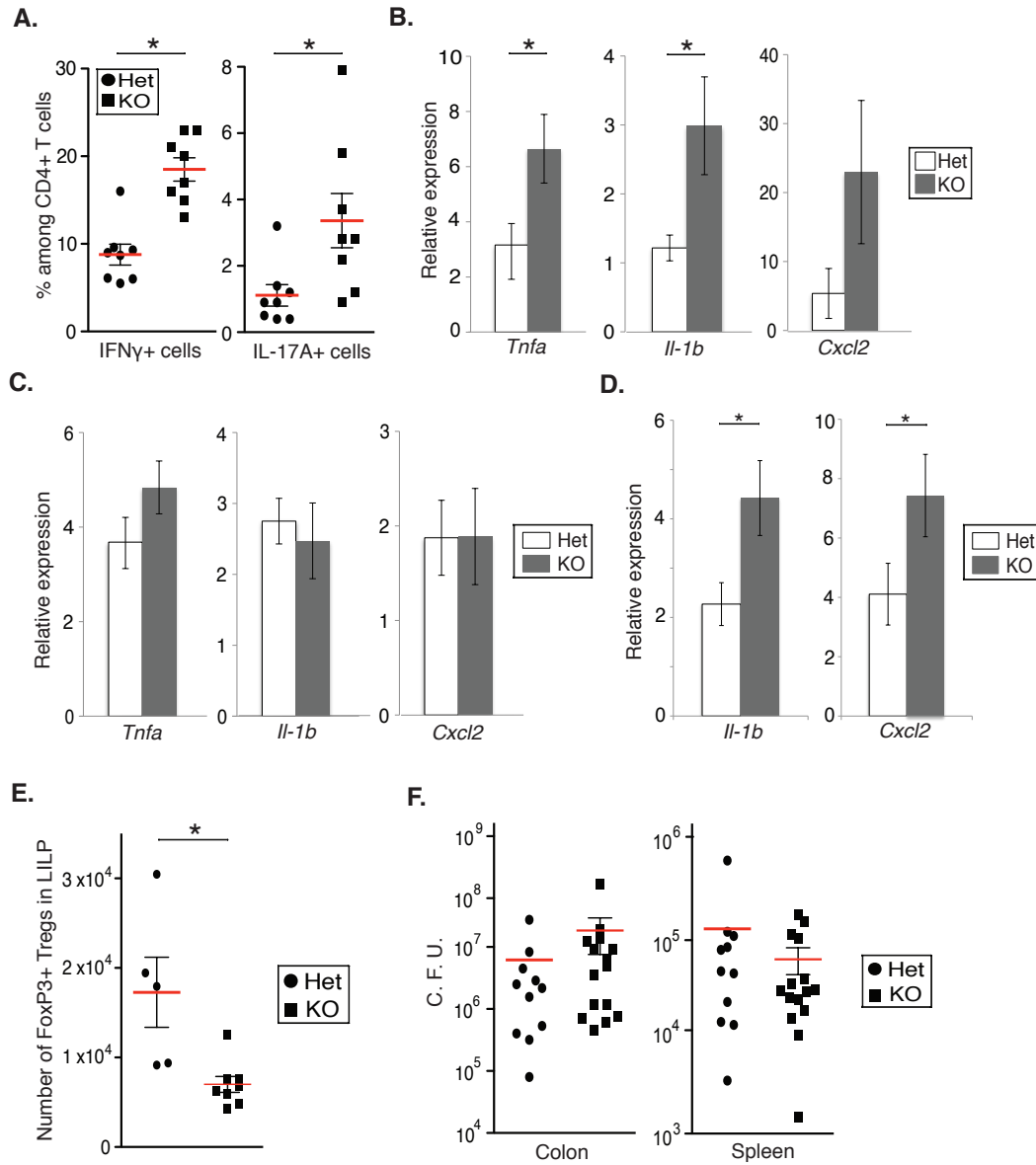


Fig. S10. Influence of GPR15 on cytokine production during inflammation and on Treg cell function. (A) Percentage of IFN γ ⁺ or IL-17⁺ CD4⁺ T cells in the LILP at steady state in *Gpr15*^{gfp/+} (Het) and *Gpr15*^{gfp/gfp} (KO) mice (9-11wks old in *B6/129SvEv* mixed background; n=8, combined from three independent experiments). (B-C) Taqman RT-PCR of inflammatory cytokines/chemokine (*Tnfα*, *Il-1β*, *Cxcl2*) in the LILP (B) and spleen (C) at day 3 after injection of αCD40 (FGK 45) antibody (200-300μg) into Het and KO mice. Relative expression level was normalized to *Beta-actin* (n=4-5). (D-F) Results of infection of mice with 8 x 10⁹ *C. rodentium* by gavage. (D) Taqman RT-PCR of inflammatory cytokine/chemokine (*Il-1β*, *Cxcl2*) in colon at day 10 after infection, normalized to *Beta-actin* (n=11). (E) FOXP3⁺ regulatory T cell numbers in LILP at day 10 (n=5-8). (F) Bacterial colony forming units/g weight of colon and spleen at day 9 (n=11-15).

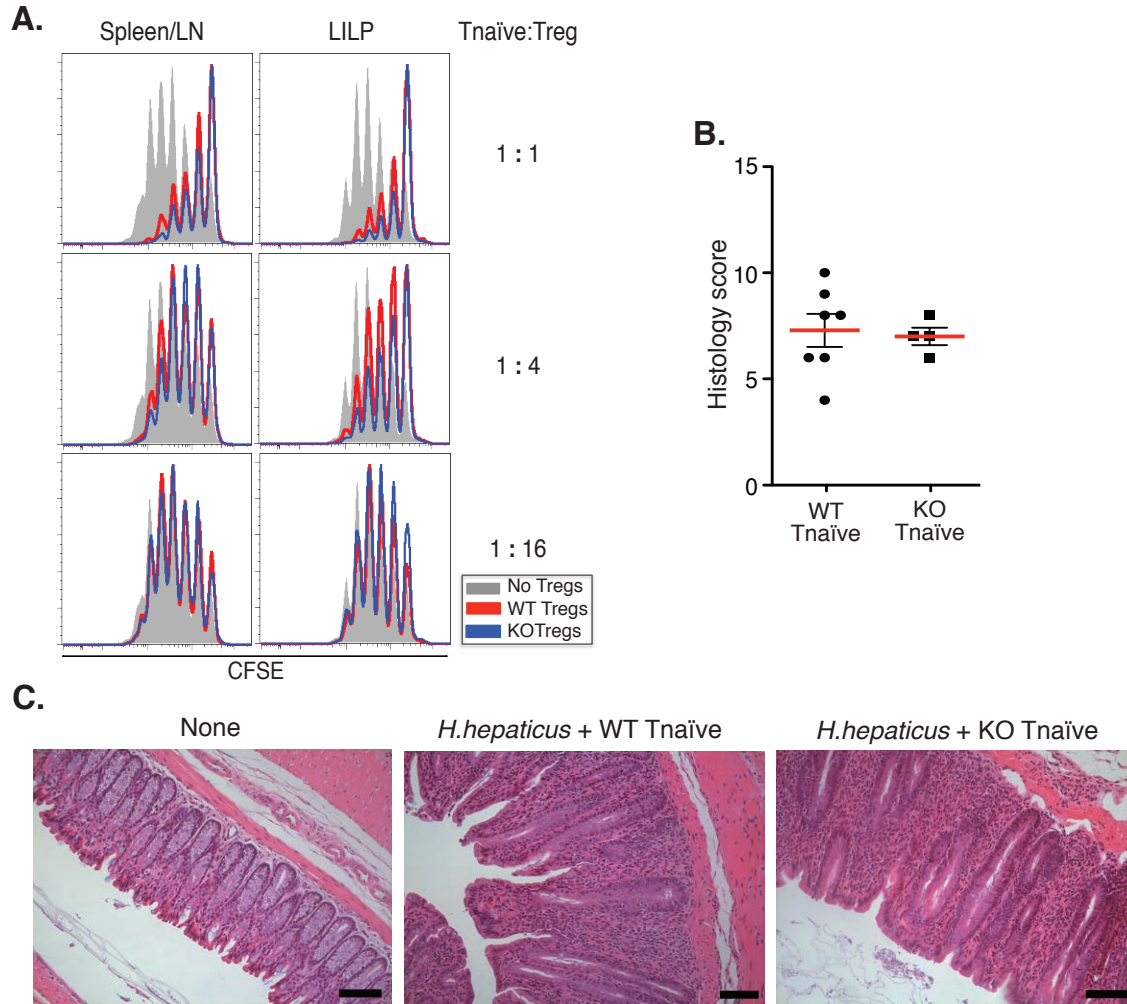


Fig. S11. Tregs and T_{naïve} cells from *Gpr15* KO mice function as well as their counterparts from WT mice in vitro and in vivo, respectively. **(A)** FOXP3/mRFP⁺ regulatory T cells in spleen and lymph nodes or LILP from *Foxp3^{ires-mrfp}* and *Foxp3^{ires-mrfp} Gpr15^{gfp/gfp}* mice were sorted and tested for their suppressive activity in vitro. **(B-C)** *H. hepaticus*-infected *Rag2^{-/-}* mice received 3×10^5 CD4⁺ T_{naïve} cells transferred either from *Foxp3^{ires-mrfp}* mice or *Gpr15^{gfp/gfp} Foxp3^{ires-mrfp}* mice. **(B)** Histology scores (n=4-7). **(C)** H&E staining of distal colon section of either *Rag2^{-/-}* without any treatment (None), with wild-type T_{naïve} transfer, or with KO T_{naïve} transfer (Bar=70μm). * p<0.05 (t-test).

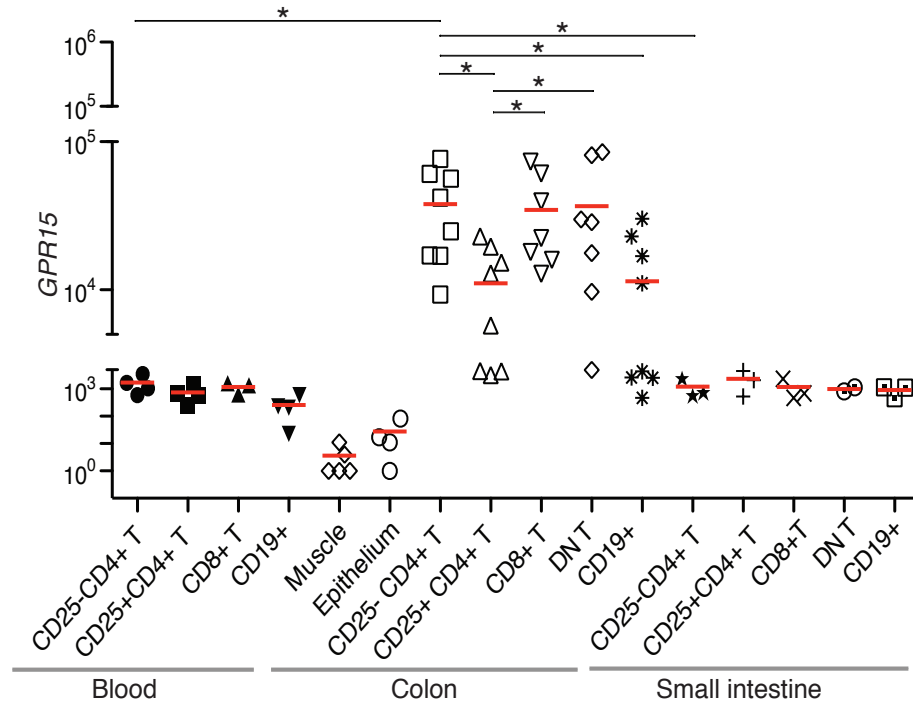


Fig. S12. Expression of *GPR15* mRNA in cells from human tissues. Lymphocytes were sorted from human blood and tissues and Taqman RT-PCR was performed for *GPR15* and normalized using *BETA-ACTIN*. (Blood: n=4, Colon: n=4-8, Small intestine: n=3 (1 from duodenum and 2 from ileum)). * $p < 0.05$ (t-test).

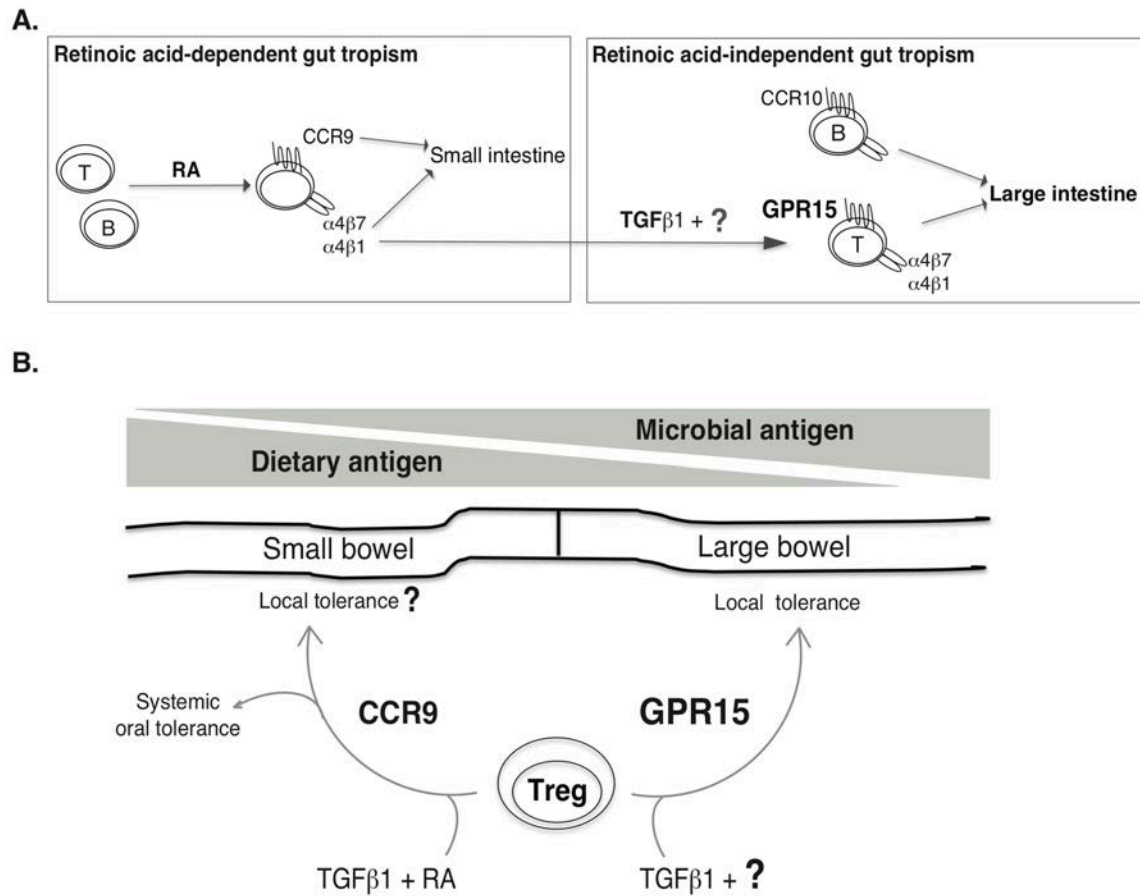


Fig. S13. Model for regulation of lymphocyte trafficking to the small versus large intestine. **(A)** Retinoic acid is well known to induce tropism for the small intestine by inducing integrin $\alpha 4$ and CCR9 expression in T and B cells (21, 34, 35). According to our study, there is an additional retinoic acid-independent pathway to induce tropism for the large intestine. TGF- β combined with unknown factors has a role in inducing GPR15 expression in vivo to enable T cells to home to the large intestine. Integrin $\alpha 4$ is still required for the majority of GPR15-mediated homing to the large intestine. Therefore, homing of lymphocytes to small and large intestine is controlled differentially by at least two different cues and by distinct homing receptors. **(B)** According to our study, adaptive immune responses in the gut are compartmentalized between the small bowel and the large bowel. Therefore, immune tolerance in the gut is also differentially regulated in those two regions. TGF- β appears to be crucial for Treg-mediated tolerance in both cases either for differentiation of peripherally derived Tregs or for induction of GPR15 for the large intestine. While CCR9 is important for the induction of systemic oral tolerance to food antigens (36), its role for Treg homing during the local immune regulation in the small intestine is not known.

Table S1. Affymetrix gene chip analysis between GFP⁺ and GFP⁻ CD4⁺ T cells from LILP of *Gpr15*^{gfp/+} mice.

Systematic	Gene Title	Gene Symbol	Fold change	Ttest p	Genbank
1418365_at	cathepsin H	Ctsh	15.23	0.04	NM_007801
1437250_at	melanoregulin	Mreg	12.31	0.03	AV298358
1421957_a_at	phosphate cytidylyltransferase 1, choline, alpha isoform	Pcyt1a	9.38	0.04	NM_009981
1428034_a_at	tumor necrosis factor receptor superfamily, member 9	Tnfrsf9	7.85	0.05	BC028507
1433933_s_at	solute carrier organic anion transporter family, member 2b1	Slco2b1	7.65	0.05	BB553107
1425546_a_at	transferrin	Trf	6.53	0.04	AF440692
1451318_a_at	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog /// similar to Yam	Lyn /// LOC676654	6.24	0.02	M57697
1447541_s_at	integrin, alpha E, epithelial-associated	Itgae	6.17	0.01	AV210813
1420765_a_at	forkhead box P3	Foxp3	5.98	0.00	NM_054039
1451776_s_at	homeobox only domain	Hod	5.65	0.05	BC024546
1431296_at	G protein-coupled receptor 15	Gpr15	5.54	0.05	AA555873
1449216_at	integrin, alpha E, epithelial-associated	Itgae	4.97	0.03	NM_008399
1438365_x_at	lysosomal-associated protein transmembrane 4B	Laptm4b	4.55	0.01	BB560429
1426750_at	filamin, beta	Flnb	4.34	0.02	AW538200
1432669_at	RIKEN cDNA 9030420N05 gene	9030420N05Rik	4.05	0.04	AK018521
1434225_at	SWA-70 protein	Swap70	4.03	0.00	AV024531
1446957_s_at	cDNA sequence BC004022	BC004022	3.36	0.03	C81621
1428662_a_at	homeobox only domain	Hod	3.33	0.01	AK009007
1438011_at	phosphate cytidylyltransferase 1, choline, alpha isoform	Pcyt1a	3.31	0.01	BB280291
1449984_at	chemokine (C-X-C motif) ligand 2	Cxcl2	3.19	0.04	NM_009140
1428484_at	oxysterol binding protein-like 3	Osbpl3	3.18	0.01	AK004768
1437868_at	cDNA sequence BC023892	BC023892	3.06	0.02	BE687858
1455805_x_at	coiled-coil domain containing 22	Ccdc22	2.92	0.03	BB165451
1442402_at	SH3 domain containing ring finger 1	Sh3rf1	2.89	0.01	BB110728
1434881_s_at	potassium channel tetramerisation domain containing 12	Kctd12	2.87	0.01	BM220945
1456609_at	calcium/calmodulin-dependent protein kinase II inhibitor 1	Camk2n1	2.84	0.00	BE994488
1417654_at	syndecan 4	Sdc4	2.74	0.03	BC005679
1438274_at	IKAROS family zinc finger 4	Ikzf4	2.72	0.03	BG071647
1418829_a_at	enolase 2, gamma neuronal	Eno2	2.49	0.04	NM_013509
1424316_at	solute carrier family 25 (mitochondrial deoxynucleotide carrier), member	Slc25a19	2.41	0.00	BC018167
1450330_at	interleukin 10	Il10	2.39	0.02	NM_010548
1439189_at	RIKEN cDNA D630023B12 gene	D630023B12Rik	2.32	0.03	BB498793
1432678_at	integrin alpha V	Itgav	2.30	0.00	AK011583
1455320_at	---	---	2.30	0.03	BQ176847
1446950_at	Thymocyte selection-associated HMG box gene	Tox	2.22	0.02	BM124834
1445612_at	---	---	2.21	0.05	AI114898
1425145_at	interleukin 1 receptor-like 1	Il1rl1	2.10	0.03	D13695
1419339_at	neuraminidase 3	Neu3	2.08	0.04	NM_016720
1435645_at	monocyte to macrophage differentiation-associated /// similar to monocy	Mmd /// LOC676546	2.08	0.05	AA472735
1425871_a_at	Single chain antibody ScFv	---	2.03	0.03	AB007986
1420692_at	interleukin 2 receptor, alpha chain	Il2ra	1.99	0.04	AF054581
1429413_at	carboxypeptidase M	Cpm	1.98	0.03	AK017670
1459219_at	Rap guanine nucleotide exchange factor (GEF) 2	Rapgef2	1.97	0.03	BM120546
1438295_at	Glucocorticoid induced transcript 1	Glicc1	1.91	0.05	BM247146
1420351_at	tumor necrosis factor receptor superfamily, member 4	Tnfrsf4	1.89	0.01	NM_011659
1442107_at	filamin, beta	Flnb	1.89	0.00	BM218614
1418154_at	cDNA sequence BC004022	BC004022	1.84	0.02	NM_030563
1457670_s_at	lamin A	Lmna	1.84	0.02	AV238225
1444706_at	RIKEN cDNA E430014L09 gene	E430014L09Rik	1.83	0.03	BB527432
1416871_at	a disintegrin and metallopeptidase domain 8	Adam8	1.78	0.03	NM_007403
1435251_at	sorting nexin 13	Snx13	1.77	0.03	AV377013
1422567_at	niban protein	Niban	1.75	0.03	NM_022018
1435981_at	10 days neonate cerebellum cDNA, RIKEN full-length enriched library, c	---	1.72	0.03	BM118398
1428509_at	myosin IE	Myo1e	1.70	0.01	AK018649
1420697_at	solute carrier family 15, member 3	Slc15a3	1.63	0.00	NM_023044
1428074_at	transmembrane protein 158	Tmem158	1.62	0.01	BE981853
1435787_at	protein phosphatase 1 (formerly 2C)-like	Ppm1l	1.61	0.02	BB035578
1448670_at	ubiquitin-conjugating enzyme E2E 3, UBC4/5 homolog (yeast)	Ube2e3	1.60	0.04	AW120830
1441033_at	transmembrane and tetratricopeptide repeat containing 2	Tmtc2	1.60	0.01	BB667269
1457644_s_at	chemokine (C-X-C motif) ligand 1	Cxcl1	1.58	0.02	BB554288
1434910_at	RIKEN cDNA A830080D01 gene	A830080D01Rik	1.57	0.00	BE136476
1425472_a_at	lamin A	Lmna	1.56	0.05	BC015302
1444426_at	RIKEN cDNA F730031O20 gene	F730031O20Rik	1.56	0.05	BB327547
1457548_at	A disintegrin-like and metallopeptidase (repolysin type) with thrombospc	Adamts6	1.56	0.02	BB227648
1419209_at	chemokine (C-X-C motif) ligand 1	Cxcl1	1.55	0.02	NM_008176
1456956_at	IKAROS family zinc finger 2	Ikzf2	1.55	0.00	BB291816
1425492_at	bone morphogenetic protein receptor, type 1A	Bmpr1a	1.54	0.00	BM939768
1416216_at	RalBP1 associated Eps domain containing protein	Reps1	1.52	0.05	NM_009048
1425493_at	bone morphogenetic protein receptor, type 1A	Bmpr1a	1.51	0.04	BM939768
1418500_at	nucleosome assembly protein 1-like 3	Nap1l3	1.46	0.04	NM_138742
1423626_at	dystonin	Dst	1.45	0.02	BB150886
1415961_at	integral membrane protein 2C	Itm2c	1.43	0.02	NM_022417
1460469_at	tumor necrosis factor receptor superfamily, member 9	Tnfrsf9	1.42	0.04	BM250782
1421375_a_at	S100 calcium binding protein A6 (calcyclin)	S100a6	1.41	0.03	NM_011313

Systematic	Gene Title	Gene Symbol	Fold change	Ttest p	Genbank
1455665_at	LON peptidase N-terminal domain and ring finger 1 /// similar to CG3236	Lonrf1 /// LOC631639	1.38	0.01	BB705689
1454702_at	RIKEN cDNA 4930503L19 gene	4930503L19Rik	1.34	0.03	AI450962
1416418_at	gamma-aminobutyric acid (GABA(A)) receptor-associated protein-like 1	Gabarapl1	1.31	0.01	AF180518
1418133_at	B-cell leukemia/lymphoma 3	Bcl3	1.31	0.03	NM_033601
1451584_at	hepatitis A virus cellular receptor 2	Havcr2	1.30	0.02	AF450241
1429400_at	chloride channel 5	Clcn5	1.30	0.01	BB794830
1419942_at	Sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	1.28	0.00	AW488194
1460700_at	signal transducer and activator of transcription 3	Stat3	1.27	0.01	AK004083
1423488_at	monocyte to macrophage differentiation-associated	Mmd	1.23	0.01	BC021914
1440959_s_at	myoneurin	Mynn	1.19	0.00	BB759556
1417162_at	transmembrane BAX inhibitor motif containing 1	Tmbim1	1.18	0.05	BC004752
1435885_s_at	intersectin 1 (SH3 domain protein 1A)	Itn1	1.18	0.00	BM248471
1447211_at	nuclear receptor interacting protein 1	Nrip1	1.17	0.04	BE956701
1434302_at	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	Raph1	1.13	0.03	AV307311
1437467_at	activated leukocyte cell adhesion molecule	Alcam	1.12	0.05	AV315205
1420895_at	transforming growth factor, beta receptor I	Tgfb1	1.11	0.05	BM248342
1426875_s_at	sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	1.06	0.00	BM210600
1454701_at	RIKEN cDNA 4930503L19 gene	4930503L19Rik	1.05	0.02	AI450962
1434310_at	bone morphogenic protein receptor, type II (serine/threonine kinase)	Bmpr2	1.05	0.05	AW546137
1449835_at	programmed cell death 1	Pdcd1	1.03	0.05	NM_008798
1437404_at	microtubule associated serine/threonine kinase family member 4	Mast4	1.03	0.05	AI642422
1444273_at	expressed sequence AW555355	AW555355	1.01	0.02	AW555355
1426965_at	RAS related protein 2a	Rap2a	0.99	0.02	BC025198
1455030_at	protein tyrosine phosphatase, receptor type, J	Ptpnj	0.99	0.01	AI116234
1444299_at	RIKEN cDNA A430093F15 gene	A430093F15Rik	0.99	0.05	BB209605
1431293_a_at	claudin domain containing 1	Cldnd1	0.96	0.05	AK012260
1416419_s_at	gamma-aminobutyric acid (GABA(A)) receptor-associated protein-like 1	Gabarapl1	0.94	0.05	AF180518
1446389_at	Nuclear receptor interacting protein 1	Nrip1	0.94	0.03	AW553331
1456810_at	vacuolar protein sorting 54 (yeast)	Vps54	0.93	0.04	BB468447
1454777_at	solute carrier organic anion transporter family, member 2b1	Slco2b1	0.92	0.04	BB553107
1435703_at	Transcribed locus	---	0.91	0.03	AW045947
1426063_a_at	GTP binding protein (gene overexpressed in skeletal muscle)	Gem	0.90	0.01	U10551
1445641_at	ELOVL family member 6, elongation of long chain fatty acids (yeast)	Elov16	0.89	0.01	BB727879
1438701_at	bicaudal D homolog 1 (Drosophila)	Bicd1	0.88	0.01	BB130665
1453375_at	RIKEN cDNA 4930422N03 gene	4930422N03Rik	0.88	0.04	BB071620
1437399_at	claudin domain containing 1	Cldnd1	0.88	0.01	BE627927
1456174_x_at	N-myc downstream regulated gene 1	Ndr1	0.84	0.00	AV309418
1427892_at	myosin IG	Myo1g	0.84	0.00	BB235320
1455009_at	carboxypeptidase D	Cpd	0.83	0.02	AW550842
1435580_at	RIKEN cDNA C230081A13 gene	C230081A13Rik	0.83	0.02	AW553275
1424317_at	solute carrier family 25 (mitochondrial deoxynucleotide carrier), member	Slc25a19	0.80	0.01	BC018167
1425264_s_at	myelin basic protein	Mbp	-0.80	0.05	BB181247
1450241_a_at	ecotropic viral integration site 2a	Evi2a	-0.81	0.04	NM_010161
1424241_at	solute carrier family 30 (zinc transporter), member 6	Slc30a6	-0.81	0.00	AF233346
1441347_at	HIV-1 Rev binding protein-like	Hrbl	-0.81	0.01	BB153954
1437641_at	RIKEN cDNA 4930535B03 gene	4930535B03Rik	-0.81	0.01	BE981473
1438981_at	Protein kinase C, beta 1	Prkcb1	-0.82	0.02	BB160675
1436235_x_at	RIKEN cDNA 4732471D19 gene	4732471D19Rik	-0.83	0.04	BB750674
1427156_s_at	activating signal cointegrator 1 complex subunit 2	Ascc2	-0.83	0.05	BB756983
1433777_at	l(3)mbl-like 2 (Drosophila)	L3mbtl2	-0.84	0.05	BB152370
1422439_a_at	cyclin-dependent kinase 4 /// similar to Cell division protein kinase 4 (Cy	Cdk4 /// LOC640611	-0.85	0.01	NM_009870
1440326_at	Src family associated phosphoprotein 1	Skap1	-0.85	0.01	AV312674
1423317_at	RIKEN cDNA 3110001D03 gene	3110001D03Rik	-0.85	0.05	BE915283
1442494_at	expressed sequence C79242	C79242	-0.85	0.05	BG066459
1443894_at	Neurofibromatosis 1	Nf1	-0.86	0.04	BB236216
1436424_at	RIKEN cDNA 1600020E01 gene	1600020E01Rik	-0.86	0.05	BG070208
1430982_at	splicing factor, arginine/serine-rich 1 (ASF/SF2)	Sfrs1	-0.87	0.04	BF682801
1428113_at	transmembrane and tetratricopeptide repeat containing 4	Tmtc4	-0.88	0.01	BB278364
1441145_at	RIKEN cDNA D030065N23 gene	D030065N23Rik	-0.88	0.01	BB448266
1455370_at	RIKEN cDNA A630023P12 gene	A630023P12Rik	-0.89	0.05	AI451630
1423924_s_at	tetraspanin 14	Tspan14	-0.89	0.01	BC025568
1460002_at	tousled-like kinase 1	Tlk1	-0.89	0.04	BM244995
1424474_a_at	calcium/calmodulin-dependent protein kinase 2, beta	Camk2	-0.90	0.04	BI157430
1418893_at	pre B-cell leukemia transcription factor 2	Pbx2	-0.90	0.01	NM_017463
1415916_a_at	methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methen	Mthfd1	-0.91	0.03	NM_138745
1426710_at	calmodulin 3	Calm3	-0.91	0.03	BB396904
1420401_a_at	receptor (calcitonin) activity modifying protein 3	Ramp3	-0.92	0.04	NM_019511
1436508_at	RIKEN cDNA 2410014A08 gene	2410014A08Rik	-0.92	0.00	BB000110
1438172_x_at	exonuclease domain containing 1	Exod1	-0.92	0.01	BB091183
1435822_at	RIKEN cDNA D830012I24 gene	D830012I24Rik	-0.93	0.04	BB318743
1428302_at	mitochondrial ribosomal protein L48	Mrpl48	-0.94	0.05	BG064141
1435695_a_at	RIKEN cDNA A030007L17 gene	A030007L17Rik	-0.94	0.01	AA673177
1442185_at	---	---	-0.94	0.05	AV382148
1458684_at	synovial sarcoma translocation, Chromosome 18	Ss18	-0.95	0.01	BG065620
1425014_at	nuclear receptor subfamily 2, group C, member 2	Nr2c2	-0.95	0.02	AU066920

Systematic	Gene Title	Gene Symbol	Fold change	Ttest p	Genbank
1448686_at	interleukin 16	Il16	-0.96	0.05	BC026894
1455353_at	transmembrane and coiled coil domains 1	Tmcc1	-0.96	0.00	AV221889
1451567_a_at	interferon activated gene 203	Ifi203	-0.97	0.03	BC008167
1438391_x_at	hydroxysteroid (17-beta) dehydrogenase 10	Hsd17b10	-0.97	0.05	AV078914
1460555_at	RIKEN cDNA 6330500D04 gene	6330500D04Rik	-0.98	0.03	BM242294
1428390_at	WD repeat domain 43	Wdr43	-0.98	0.05	AK012043
1442254_at	Transcribed locus	---	-0.98	0.03	BB366659
1456432_at	GRB2-related adaptor protein 2	Grap2	-0.98	0.04	BB168280
1417315_at	GRIP1 associated protein 1	Gripap1	-0.98	0.00	BG864756
1419033_at	---	---	-0.99	0.01	AW556821
1435331_at	expressed sequence AI447904	AI447904	-0.99	0.01	BM241008
1416522_a_at	gene rich cluster, C10 gene	Grcc10	-1.00	0.03	NM_013535
1439571_at	RIKEN cDNA E230008J23 gene	E230008J23Rik	-1.00	0.00	BB820889
1425270_at	kinesin family member 1B	Kif1b	-1.00	0.05	BE199508
1426342_at	STT3, subunit of the oligosaccharyltransferase complex, homolog B (S. cerevisiae)	Stt3b	-1.00	0.04	AK018758
1426813_at	LTV1 homolog (S. cerevisiae)	Ltv1	-1.01	0.00	U01139
1449303_at	sestrin 3	Sesn3	-1.01	0.01	NM_030261
1450966_at	carnitine O-octanoyltransferase	Crot	-1.01	0.01	BB283187
1416170_at	TNF receptor-associated protein 1	Trap1	-1.01	0.02	NM_026508
1436212_at	transmembrane protein 71	Tmem71	-1.03	0.02	AV173260
1420950_at	zinc and ring finger 1	Znrf1	-1.03	0.02	BB026596
1460419_a_at	protein kinase C, beta 1	Prkcb1	-1.04	0.02	X59274
1428392_at	Ras association (RalGDS/AF-6) domain family 2	Rassf2	-1.04	0.05	AK018504
1427342_at	FAST kinase domains 1	Fastkd1	-1.04	0.03	BC023501
1418968_at	RB1-inducible coiled-coil 1	Rb1cc1	-1.04	0.02	BE570980
1449855_s_at	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase) /// ubiquitin	Uchl3 /// Uchl4	-1.05	0.02	AB033370
1428233_at	cleavage and polyadenylation specific factor 6	Cpsf6	-1.05	0.04	BB425379
1428322_a_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	Ndufb10	-1.05	0.04	BI905689
1424181_at	septin 6	5-Sep	-1.05	0.03	BC010489
1452470_at	centrosomal protein 350		-1.06	0.01	BC019716
1418495_at	zinc finger CCCH type containing 8	Zc3h8	-1.06	0.00	NM_020594
1431054_at	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae)	Lsm6	-1.07	0.05	AK019126
1435655_at	small nucleolar RNA, H/ACA box 65	Snora65	-1.08	0.02	BG807990
1420876_a_at	septin 6	5-Sep	-1.08	0.03	NM_019942
1431981_at	hypoxia inducible factor 1, alpha subunit		-1.09	0.03	AK017853
1421819_a_at	SET translocation /// similar to SET protein (Phosphatase 2A inhibitor I2F)	Set /// LOC671392	-1.10	0.02	BF134272
1448864_at	SNF related kinase	Snrk	-1.11	0.01	NM_133741
1416906_at	anaphase-promoting complex subunit 5	Anapc5	-1.11	0.03	NM_021505
1435329_at	F-box and leucine-rich repeat protein 11	Fbxl11	-1.12	0.02	BE690994
1435925_at	G protein-coupled receptor kinase-interactor 2	Git2	-1.13	0.01	BB377392
1423775_s_at	protein regulator of cytokinesis 1	Prc1	-1.13	0.03	BC005475
1458038_at	---	---	-1.14	0.01	BG063073
1433596_at	DnaJ (Hsp40) homolog, subfamily C, member 6	Dnajc6	-1.14	0.04	BQ175337
1423241_a_at	transcription factor Dp 1 /// similar to Transcription factor Dp-1 (E2F dim	Tfdp1 /// LOC664889	-1.15	0.04	BG075396
1434301_at	RIKEN cDNA D330050I23 gene	D330050I23Rik	-1.16	0.03	BE303700
1430127_a_at	cyclin D2	Ccnd2	-1.19	0.01	AK007904
1454920_at	ubiquitin-like, containing PHD and RING finger domains 2	Uhrf2	-1.19	0.05	BQ266387
1445895_at	T-cell receptor beta, variable 8.2	Tcrb-V8.2	-1.20	0.05	AI450773
1456678_at	Src family associated phosphoprotein 1	Skap1	-1.20	0.04	AV314270
1432850_at	RIKEN cDNA 5430434G16 gene	5430434G16Rik	-1.21	0.02	AK017390
1444203_at	Transcribed locus	---	-1.21	0.05	AI661342
1420381_a_at	ribosomal protein L31	Rpl31	-1.21	0.01	NM_053257
1419119_at	hematopoietic cell signal transducer	Hcst	-1.22	0.01	AF172930
1443279_at	Nemo like kinase	Nlk	-1.22	0.04	BB204492
1418181_at	protein tyrosine phosphatase 4a3	pPtp4a3	-1.23	0.00	AK014601
1417384_at	ectonucleoside triphosphate diphosphohydrolase 5	Entpd5	-1.23	0.03	NM_007647
1454899_at	LIM domain containing preferred translocation partner in lipoma	Lpp	-1.26	0.02	BB089138
1437709_x_at	tetratricopeptide repeat domain 13	Ttc13	-1.26	0.04	BB492914
1433582_at	RIKEN cDNA 1190002N15 gene	1190002N15Rik	-1.27	0.03	AV309085
1442332_at	Transforming growth factor, beta receptor III	Tgfb3	-1.28	0.05	BG794571
1420634_a_at	MAD homolog 2 (Drosophila)	Smad2	-1.28	0.01	NM_010754
1458031_at	Solute carrier family 25, member 40	Slc25a40	-1.28	0.05	BG075632
1423928_at	phosphoglycerate dehydrogenase like 1	Phgdhl1	-1.30	0.00	BC024467
1441164_at	Phosphatidylinositol-4-phosphate 5-kinase, type II, beta	Pip5k2b	-1.31	0.02	BG071985
1427135_at	splicing factor, arginine/serine-rich 12	Sfrs12	-1.31	0.01	AV012790
1454654_at	disrupted in renal carcinoma 2 (human)	Dirc2	-1.32	0.04	BG069395
1423608_at	integral membrane protein 2A	Itm2a	-1.33	0.04	BI966443
1434544_at	bolA-like 2 (E. coli)	Bola2	-1.33	0.01	BE992311
1426002_a_at	cell division cycle 7 (S. cerevisiae)	Cdc7	-1.38	0.01	AB018574
1434105_at	EPM2A (laforin) interacting protein 1	Epm2aip1	-1.38	0.04	AV340515
1418641_at	lymphocyte cytosolic protein 2	Lcp2	-1.39	0.02	BC006948
1434532_at	cDNA sequence BC035295	BC035295	-1.40	0.00	BB796273
1456655_at	Exostoses (multiple) 1	Ext1	-1.40	0.05	BM231698
1439449_at	special AT-rich sequence binding protein 1	Satb1	-1.41	0.01	BB724383
1418826_at	membrane-spanning 4-domains, subfamily A, member 6B	Ms4a6b	-1.42	0.01	NM_027209

Systematic	Gene Title	Gene Symbol	Fold change	Ttest p	Genbank
1448274_at	complement component 1, q subcomponent binding protein	C1qbp	-1.42	0.01	NM_007573
1423478_at	protein kinase C, beta 1	Prkcb1	-1.42	0.05	BF660388
1452676_a_at	polyribonucleotide nucleotidyltransferase 1	Pnpt1	-1.43	0.03	BB777815
1455711_at	deltex 4 homolog (Drosophila)	Dtx4	-1.44	0.04	AW122183
1443263_at	BTB and CNC homology 2	Bach2	-1.46	0.02	AV365508
1458406_at	Expressed sequence AI429294	AI429294	-1.47	0.00	BG144063
1456433_at	regulator of chromosome condensation (RCC1) and BTB (POZ) domain	Rcbb1	-1.47	0.01	BB000798
1450095_a_at	acylphosphatase 1, erythrocyte (common) type	Acyp1	-1.48	0.04	NM_025421
1438476_a_at	chromodomain helicase DNA binding protein 4	Chd4	-1.49	0.04	BB201828
1427184_at	T-cell receptor beta, variable 13	Tcrb-V13	-1.51	0.05	BF318536
1417136_s_at	serine/arginine-rich protein specific kinase 2	Srp2	-1.51	0.03	NM_009274
1417509_at	ring finger protein (C3HC4 type) 19	Rnf19	-1.52	0.01	AF120206
1452151_at	cDNA sequence BC021523	BC021523	-1.55	0.04	BC021523
1454745_at	Rho GTPase activating protein 29	Arhgap29	-1.58	0.04	BG074320
1417164_at	dual specificity phosphatase 10	Dusp10	-1.58	0.03	NM_022019
1423756_s_at	insulin-like growth factor binding protein 4	Igfbp4	-1.64	0.02	BC019836
1455132_at	RIKEN cDNA A430107D22 gene	A430107D22Rik	-1.64	0.03	AV312663
1421305_x_at	rabaptin, RAB GTPase binding effector protein 1	Rabep1	-1.65	0.01	NM_019400
1416697_at	dipeptidylpeptidase 4	Dpp4	-1.66	0.01	NM_010074
1434036_at	metastasis suppressor 1	Mtss1	-1.68	0.04	AV024771
1424464_s_at	RIKEN cDNA 2210010L05 gene	2210010L05Rik	-1.68	0.04	BF225441
1447092_at	Platelet/endothelial cell adhesion molecule 1	Pecam1	-1.69	0.03	BG063222
1419163_s_at	DnaJ (Hsp40) homolog, subfamily C, member 3	Dnajc3	-1.70	0.04	BE624323
1424826_s_at	metastasis suppressor 1	Mtss1	-1.71	0.02	BC024131
1417236_at	EH-domain containing 3	Ehd3	-1.74	0.03	BM234719
1426505_at	ecotropic viral integration site 2b	Evi2b	-1.74	0.01	AI122415
1426850_a_at	mitogen activated protein kinase kinase 6	Map2k6	-1.75	0.05	BB261602
1441705_at	expressed sequence AU015680	AU015680	-1.76	0.00	BG145559
1426892_at	utrophin	Utrn	-1.76	0.04	AI788797
1418222_at	RIKEN cDNA 2610024G14 gene	2610024G14Rik	-1.76	0.01	NM_019836
1456121_at	leucine-rich repeats and IQ motif containing 2	Lrriq2	-1.76	0.05	BM224149
1456358_at	Ets variant gene 3	Etv3	-1.79	0.05	BM932547
1430191_at	RIKEN cDNA 9130004J05 gene	9130004J05Rik	-1.80	0.02	BB748887
1426343_at	STT3, subunit of the oligosaccharyltransferase complex, homolog B (S. cerevisiae)	Stt3b	-1.81	0.00	AK018758
1454578_at	RIKEN cDNA 6030458A19 gene	6030458A19Rik	-1.84	0.01	AK020073
1443090_at	Choline/ethanolaminephosphotransferase 1	Cept1	-1.86	0.01	BB361936
1441068_at	RIKEN cDNA A130001G05 gene	A130001G05Rik	-1.86	0.01	BB631473
1449619_s_at	Rho GTPase activating protein 9	Arhgap9	-1.91	0.03	AU043488
1424505_at	required for meiotic nuclear division 1 homolog (S. cerevisiae)	Rmnd1	-1.93	0.01	BC027299
1428800_a_at	pseudouridylate synthase 7 homolog (S. cerevisiae)-like	Pus7l	-1.93	0.02	AK019372
1453571_at	DEP domain containing 6	Depdc6	-1.96	0.04	BB324973
1436097_x_at	Rho GTPase activating protein 9	Arhgap9	-2.10	0.04	BB327418
1417663_a_at	N-myc downstream regulated gene 3	Ndr3	-2.13	0.00	BE631549
1448208_at	MAD homolog 1 (Drosophila)	Smad1	-2.18	0.01	NM_008539
1445028_at	Protein kinase C, alpha	Prkca	-2.20	0.04	BE993069
1419361_at	synovial sarcoma translocation, Chromosome 18	Ss18	-2.21	0.04	AI528781
1417235_at	EH-domain containing 3	Ehd3	-2.23	0.05	BM234719
1437584_at	Cyclin D3	Ccnd3	-2.31	0.00	BE685667
1450639_at	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	Slc28a2	-2.37	0.01	NM_021520
1423313_at	phosphodiesterase 7A	Pde7a	-2.39	0.02	BG070255
1457687_at	B-cell leukemia/lymphoma 2	Bcl2	-2.42	0.00	BI664467
1438125_at	RIKEN cDNA C230085N15 gene	C230085N15Rik	-2.43	0.03	BB393897
1433944_at	HECT domain containing 2	Hectd2	-2.45	0.05	AV256030
1426044_a_at	protein kinase C, theta	Prkcq	-2.55	0.03	AB062122
1416318_at	serine (or cysteine) peptidase inhibitor, clade B, member 1a	Serpib1a	-2.70	0.00	AF426024
1433169_at	RIKEN cDNA 5830456J23 gene	5830456J23Rik	-2.70	0.03	AK018017
1427683_at	early growth response 2	Egr2	-2.84	0.02	X06746
1427417_at	sex comb on midleg-like 4 (Drosophila)	Scml4	-2.84	0.04	BB212066
1425628_a_at	similar to General transcription factor II-I (GTFII-I) (TFII-I) (Bruton tyrosinase)	LOC669007	-2.86	0.02	AF043220
1430129_a_at	COMM domain containing 8	Comm8	-3.28	0.05	AK017777
1440842_at	RIKEN cDNA C230085N15 gene	C230085N15Rik	-3.31	0.04	AI449439
1433795_at	transforming growth factor, beta receptor III	Tgfb3	-3.33	0.01	BM122301
1430622_at	RIKEN cDNA 4833423F13 gene	4833423F13Rik	-3.35	0.02	AW764291
1437699_at	RIKEN cDNA E430014B02 gene	E430014B02Rik	-3.65	0.03	BB031353
1458218_s_at	phosphodiesterase 7A	Pde7a	-3.70	0.00	AU015378
1452416_at	interleukin 6 receptor, alpha	Il6ra	-3.83	0.03	X53802
1449235_at	Fas ligand (TNF superfamily, member 6)	Fasli	-3.83	0.02	NM_010177
1427819_at	---	---	-5.55	0.03	BC027249
1443579_s_at	DEP domain containing 6	Depdc6	-5.76	0.03	AI957118
1452792_at	DAZ interacting protein 1	Dzip1	-6.14	0.01	AI509011
1430448_at	RIKEN cDNA 6720418B01 gene	6720418B01Rik	-6.62	0.01	BB392953
1433939_at	hypothetical protein A730046J16	A730046J16	-6.96	0.02	BQ177036
1441172_at	AF4/FMR2 family, member 3	Aff3	-7.40	0.01	BM239026
1433977_at	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	Hs3st3b1	-12.09	0.01	BG918344
1451363_a_at	DENN/MADD domain containing 2D	Dennd2d	-13.02	0.03	BC008266